

Notes from the 10th International Conference on Harmful Algal Blooms

provided by Dave Buzan and Tracy Villareal

Texas was well represented at the conference. Tracy Villareal was on the organizing committee and authored and coauthored too many presentations and posters to count. Ed Buskey from UTMSI and Jay Pinckney and Lisa Campbell from TAMU-College Station also participated. Carm Tomas graciously put together a poster of golden algae occurrence in the U.S., listing Joan Glass and Jack Ralph as first and second author. Jim Simons coauthored two posters and Cindy Contreras and Kirk Wiles each coauthored one poster. Grace Chen helped prepare maps for two posters.

There were over 700 participants from about 54 countries. There were over 400 posters and nearly 200 presentations at the meeting. I have tried to summarize highlights that I thought were particularly applicable to Texas. Some deal with algae known to cause problems in Texas. Other notes deal with algae that might cause problems in Texas in the future.

Karenia brevis (dinoflagellate)

Environmental conditions:

- Texas *K. brevis* compared to Florida *K. brevis*: lower growth rates; similar light saturation; lower upper salinity tolerance (40 ppt for TX compared to 46 ppt for FL); similar salinity minimum (22 – 24 ppt); similar temperature tolerance 15-30 degrees C
- Produce mycosporine amino acids which may help protect from ultraviolet radiation.
- **Four *Karenia brevis* Blooms: A Comparative Analysis by Gabriel Vargo, et al.** Four blooms on West Florida Shelf from 1998 to 2002. “All blooms appeared after breakdown of vertical stratification, with three of the blooms associated with near shore thermal or salinity fronts after onshore transport. Nitrogen and phosphorus levels suggested probably nitrogen limitation. Dissolved silicates were measured with all four blooms. Estuarine waters containing nitrogen and phosphorus are adequate to support moderate to high populations in near shore waters.

Grazing:

- Protozoan grazers do not accumulate brevetoxins. Some protozoan grazers did not grow when fed *K. brevis* and others grew slowly. The protozoans did not selectively feed on or avoid feeding on *K. brevis*.
- *Acartia tonsa* does not feed on *K. brevis* but *K. brevis* is not toxic to *A. tonsa*

Nutrient requirements:

- Can utilize a wide variety of nitrogen sources
- **Does Nitrogen Regeneration From the Nitrogen Fixing Cyanobacteria *Trichodesmium* spp. Fuel *Karenia brevis* Blooms in the Gulf of Mexico? By Margaret Mulholland et al.** There appears to be correlation between the timing and magnitude of *K. brevis* and *Trichodesmium* blooms in the Gulf of Mexico. *Trichodesmium* fixes nitrogen and can release nitrogen that is available to other phytoplankton. Levels of inorganic and organic nitrogen accumulate around *Trichodesmium* blooms. Harpacticoid

copepods graze on *Trichodesmium* and don't form fecal pellets but release a steady stream of dissolved waste material into the water.

Toxin:

- Toxin depuration can take 2-6 weeks.
- Toxin production appears to be lower in lower temperatures and lower concentrations are found in oyster tissues during lower temperatures (Kirk Wiles).
- Clones isolated from the Texas red tide of 1999 exhibited significant differences in growth rates and toxin production.
- Aerosol concentrations were up to 5 nannograms/cubic meter during the 2000 Texas red tide (late October at the Texas State Aquarium). Aerosol particle size was about 9 microns. These particles are the size tending to be trapped in the nose, mouth and throat. The smallest particles, about 2-3 microns, can get down into the lungs.
- Toxin concentrations in oysters do not appear to be very variable. Variation less than 30 %. Four samples should be adequate to characterize shellfish toxicity in one area.
- **Florida's Red Tide Dinoflagellate, *Karenia brevis*, May Modulate its Potency by Producing a Non-toxic Competitive Antagonist by A.J. Bourdelais et al.** *K. brevis* produces 9 different toxins. The combination of toxins produced depends on the growth phase and strain of the *K. brevis* producing the toxin. It appears that *K. brevis* produces a competitive antagonist for the brevetoxin binding site. Production of this non-toxic "antagonist" which competes with the brevetoxin may explain the "rapidly decreasing potency of red tides during their terminal phases in natural environments."

Monitoring:

- **A Prototype System for Monitoring and Forecasting of Harmful Algal Blooms in the Gulf of Mexico by Richard Stumpf et al.** Satellites can detect chlorophyll changes greater than 1 microgram per liter that is about equivalent to about 100,000 *Karenia brevis* cells per liter. These are concentrations of interest because these are levels where fish mortality is expressed and respiratory irritation is experienced. Background chlorophyll tends to range between 0.1 to 0.5 micrograms per liter. Predictions were made which showed that winds blowing off shore the west FL shore were associated with more blooms of *K. brevis*. This system has had some false positives, ex. Diatom bloom was identified as a red tide, but has had few false negatives.

Control:

70% of *K. brevis* was removed during mesocosm trials with clay during the 2000 Texas red tide. A couple of questions that still need to be addressed: if insufficient clay is used the cells will be transported to the bottom but will not be held there; if the clay takes the cells to the bottom and the clay on the bottom is disturbed before it has time to compact (maybe 4 hr), the cells will be released when the clay is disturbed; right now the trials are being conducted with a phosphatic clay and it is not known if the phosphorus addition will stimulate phytoplankton growth; and future areas of research will focus on benthic impacts of the clay with the adsorbed cells.

Pfiesteria (dinoflagellate)

Toxin: There are three strains regarding toxicity: Toxic A is always actively toxic (the toxin is a water soluble ichthyotoxin and there is not a direct relationship between cell numbers and toxicity), Toxic B is temporarily toxic and Toxic C is not toxic but may physically damage fish. Toxic fraction appears to be a glycoside. *P. shumwayae* may be able to kill via micropredation causing diffuse erythema/hemorrhage, loss of cuticle, loss of scales and epidermis and fraying of fins.

Nutrients: nitrogen loading in the form of urea (probably driven by pulsed delivery of rainfall runoff) stimulates growth

Environmental conditions: 8.0-18 ppt salinity; Secchi disk <1 m; 18-26 degrees C; 6.7-13.1 mg/l oxygen; chlorophyll a > 16 micrograms/l; total P >0.01 mg/l; total Kjeldahl N > 0.5 mg/l; total dissolved N > 0.31 mg/l; particulate C > 0.25 mg/l; particulate P > 0.069 mg/l; dissolved silicate > 1.0 mg/l; ammonia > 0.04 mg/l; particulate N > 0.35 mg/l; dry years; high sediment nutrients

Distribution: global

Transport: ballast of vessels including ballast water and residual sediment and water in ballast tanks.

Prymnesium parvum

Poster: Blooms of the Ichthyotoxic Flagellate *Prymnesium parvum* in U.S. Coastal Waters: An Emerging Problem by Joan Glass, Jack Ralph and Carm Tomas et al.

While working this poster with Carm, we were approached by three researchers from Europe (one from Denmark, one from Sweden and one from Germany) who are working with *P. parvum*. The Danish researcher is investigating what *P. parvum* eats. Apparently it eats anything it can get its hands on from bacteria to algae and protozoans that are larger than it. Some organisms seem to be visibly slowed down by the toxin and then are engulfed (if equal to or smaller in size than the *P. parvum* doing the eating). In some cases the toxin causes the other organism to lyse and *P. parvum* absorbs the released goodies. They have seen several individual *P. parvum* attacking and eating a larger dinoflagellate. If concentrations of the *P. parvum* are high enough they will eat the protozoan grazers that might otherwise be eating individual *P. parvum*.

We were also approached by a Japanese researcher who has been working on *P. parvum* toxin for about the last four years and has published three papers he thought we would be interested in. I'm not certain I understood correctly but I think he said that the prymnesin toxin is the fourth largest naturally produced molecule known.

Igarashi, T., S. Aritake, and T. Yasumoto. Mechanisms underlying the hemolytic and ichthyotoxic activities of maitotoxin. Nat. Toxins, 7: 71-79 (1999).

Igarashi, T., M. Satake, and T. Yasumoto. Prymnesin-2: a potent ichthyotoxic and hemolytic glycoside isolated from the red tide alga *Prymnesium parvum*. *J. Am. Chem. Soc.*, 118: 479-480 (1996).

Igarashi, T., Y. Oshima, M. Murata, and T. Yasumoto. Chemical studies on prymnesins isolated from *Prymnesium parvum*. In: *Harmful Marine Algal Blooms*, pp. 303-308. (P. Lassus, G. Arzul, E. Erard-Le-Denn, P. Gentien, and C. Marcaillou-Le-Baut, Eds.). Paris: Lavoisier (1995).

Feeding:

- Toxin slowed down motile dinoflagellates and *P. parvum* feeding activity increased as toxicity increased.
- Feeding rate was high on nonmotile diatoms whether toxin levels were low or high
- Feeding occurs as the cell exudes pseudopodia from the posterior end of the cell. The pseudopodia encircle the food item. Sometimes two or more cells will appear to share the same large food item and it looks like one large food vacuole surrounded by several cells.
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Toxin:

- One role of toxin is to immobilize prey. Some toxic cultures have 1.6×10^9 cells per l.
- In culture, toxin concentrations are highest in the morning and then degrade during the day due to sunlight.
- All strains of *P. parvum* become toxic under reduced P concentrations. In other words, there are no nontoxic strains of *P. parvum*. They all will express toxicity when P limited.
- Low toxicity cultures are grown in a medium with plenty of N and P, no aeration, and dim light. In low toxicity cultures, the protozoan grazer, *Oxyrrhis*, will graze on *P. parvum*.
- Toxic cultures are grown in 10 ppt, N:P ratio of 80 (P limited), 15 degrees C, and 45 microEinsteins per meter squared per second of light.
- The EC₅₀ of *P. parvum* toxic cultures for the protozoan grazer, *Oxyrrhis*, is 16×10^3 *P. parvum* cells. In other words, when *P. parvum* cells reach this concentration, there is enough toxicity that they begin to kill and eat the protozoan, *Oxyrrhis*.
- Toxic to ciliates at high concentrations and did not support ciliate growth
- Cultures from North and South Carolinas exhibited relatively constant toxin levels under different nitrogen sources, over a temperature range from 20 to 30 degrees Centigrade, and over a range of salinities as low as 3 ppt.

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Control:

Sweden researchers are testing clays to control *P. parvum* because of fish kills in aquaculture along the western Swedish coast. They have been able to get partial removal with clay concentrations of 0.5 g/l. Cell removal is less than 100% (55-84%) when cells are nutrient deficient. Toxicity of cells increased after removal of clays.

Blue-green Algae

Effects:

- Reported to be respiratory, irritation of skin, mucus membranes, conjunctiva around eyes, gastrointestinal damage, pneumonia
- Palm Island Mystery disease: 140 children and 10 adults experienced severe liver, kidney and gastrointestinal system damage. Kidneys appeared most sensitive to damage. Cylindrospermopsin toxicity occurred after drinking water supply reservoir was treated with copper sulfate.
- Caruaru Dialysis Unit: Over 50 people on kidney dialysis died after exposure to microcystin from water supply reservoir.

Toxin:

- Cylindrospermopsin – an alkaloid compound. This toxin can also be produced by *Aphanizomenon ovalisporum*. The acute oral LD₅₀ is 6 mg/kg body weight. Symptoms for include pale swollen livers, fatty liver, slow acting (after 5 days) and eye damage. Cattle drinking water of 1.05 mg/l cylindrospermopsin exhibited lethargy, recumbency, death within 2 days and had pale mottled livers with distended gall bladders, hepatic necrosis and some heart damage. Toxin was not detected in cattle muscle.
- Saxitoxin produced by *Anabaena circinalis* can be removed by water treatment by maintaining pH>9 and residual chlorine greater than equal to 0.5 mg/l for 30 minutes
- Anatoxin a is a neurotoxin with an LD₅₀ of 200 micrograms/kg body weight and is produced by some *Anabaena*
- Cylindrospermopsin and Anatoxin have shown to be able to pass through drinking water treatment plants

Harmful Cyanobacterial Bloom Expansion in Estuarine and Coastal Waters: Environmental Controls and Management Options by Michael Piehler et al.

In St. John's River, FL, nitrogen fixation by cyanobacteria is always stimulated by phosphorus addition. Future nutrient management will need to include controls for both nitrogen and phosphorus. In the Neuse River, NC, a decreasing ratio of inorganic nitrogen to inorganic phosphorus stimulated nitrogen fixation by cyanobacteria. In a portion of this system, nitrogen fixation was estimated to be about 35 tons per year of nitrogen or about 3% of the total nitrogen input to that portion.

Cylindrospermopsis raciborskii: doesn't form scums, highest concentrations form at depths where it is not typically visible, water soluble toxin can be outside of cells, can be found in treated drinking water

Highest growth rates at 25 degrees C when compared to growth rates at 15 and 20 degrees C.

Rate of toxin production decreases at 25 degrees compared to rates at 15 and 20 degrees C.

Produces a hepatotoxin with a LD₅₀ of 200 micrograms/kg body weight

Lyngbya majuscula

Produces saxitoxin and dermatotoxin (debromoaplysiatoxin and Lyngbya toxin-a) as part of the over 100 bioactive compounds it is known to produce.

Causes severe contact dermatitis, asthma-like symptoms, nausea and dizziness

Blooms occur in: high light, temperatures >20 degrees C, sources of P, Fe and organic material

Chemical Ecology of Tropical Benthic Marine Cyanobacterial Blooms by Valerie Paul et al. Benthic cyanobacteria like *Lyngbya majuscula* found in coastal waters around the world can form expansive benthic layers and inhibit feeding by herbivorous fish and sea urchins. These benthic blooms usually end following a substantial physical disturbance like storm passage.

The Toxicological Impact of *Lyngbya majuscula* Upon the Rabbitfish *Siganus fuscescens* and the Sea Hares *Stylocheilus longicauda* and *Bursatella leachii* by Angela Capper et al. Rabbitfish eat other plants and algae before *Lyngbya*. *Lyngbya* can pass through the fishes' guts with still active toxins. Sea hares like to eat *Lyngbya* and can eat half to their whole weight per day. *Lyngbya* toxin was concentrated in the digestive gland and the body, not in ink, eggs or fecal matter. Sea hares may die off after eating the *Lyngbya*. In one instance, 8 dogs died after eating sea hares which had washed ashore dead.

Trichodesmium

3 species in the Gulf of Mexico

Trichodesmium theibautii occurs offshore and produces water-soluble and lipid soluble compounds inhibiting the growth of several different types of algae. In one experiment it reduced growth of *K. brevis* by 60%. Tests for saxitoxin and protein inhibitase enzyme were negative.

Trichodesmium erythraeum occurs nearshore and produces N is thought to stimulate *K. brevis* blooms.

The coast of Brazil had been known to have an incident called Tamandare Fever that appeared to be associated with *Trichodesmium*. Symptoms included respiratory irritation and other conditions and lasted 3 days.

Microcystis aeruginosa

Toxin:

- Microcystin threshold in drinking water is 1 microgram/l
- Microcystin threshold in channel catfish culture ponds is 65 micrograms/l
- Temperate clones of *M. aeruginosa* produced highest toxin levels at 27 degrees C

Heterosigma akashiwo (a raphidophyte)

Toxin:

- Not known but does produce Reactive Oxygen Species (ROS) like hydrogen peroxide. Hydrogen peroxide causes an overactive stress response in fish resulting in mucus secretion which then results in asphyxiation.
- Nitrogen-limited cultures produced hydrogen peroxide at higher rates than phosphorus-limited cultures.

Aureoumbra lagunensis (brown tide, a pelagophyte)

Distribution: Florida Bay, northern Mexico bays, some Texas bays

Environmental conditions: blooms occur only at salinities > 40 ppt

Pseudo-nitzschia (diatoms)

Can produce domoic acid which causes Amnesic Shellfish Poisoning. Is present and abundant in the northern Gulf of Mexico. Several species present. Why does it not appear to cause ASP in the Gulf? It does not occur in high numbers at low salinities. Oysters don't tend to eat it.

Pseudo-nitzschia was identified from Galveston Bay waters at least 15 years ago and was shown to be toxic.

Transport of HABs

- Ballast water of ships: 5% of all ships coming to New Zealand are transporting HABs in ballast water. 20% of all ships coming to the United Kingdom are transporting HABs in ballast water. 4 of 11 ships sampled entering Chesapeake Bay had *Pfiesteria* in the ballast water.

- Residual ballast water and sediment of ships: 7 of 24 ships sampled entering Chesapeake Bay without ballast water in 2002 had *Pfiesteria* in residual water or sediments in the ballast chambers.
- Floating plastic litter from an area with a bloom of *Alexandrium* was shown to have live cells and cysts.
- Transport of live shellfish (viable cells and cysts of HABs have been shown to survive in the guts of shellfish)
- Transport in major oceanic currents like the Gulf stream

Control of HABs

- Lysis of HAB cells by bacteria and viruses
- Consumption by acidians
- Treatment with sophorolipid, a surfactant
- Treatment with ozone
- Treatment with clay (at least one study shows impacts on mussels and scallops). **Effects of Phosphatic Clay Dispersal at a Salmon Farm in Puget Sound by J. Rensel.** They tested the use of clays in controlling algae. Perimeter skirts were placed around a large pen and the pen was treated with clay at a concentration of 0.1 gram/liter. 50-80% of the diatoms were removed. 70-90% of the microflagellates were removed. There were no significant effects on water quality or sediment.
- Treatment with clay continued: One study showed that china clay (used to treat HABs in Korea) has a significant negative impact on filter feeding benthic invertebrates and that some species are more sensitive than others.
- Grazing by certain zooplankters
- Control of nutrients, for cyanobacterial blooms maintain P below 100 micrograms/l. High frequency, several-fold increases in P lasting minutes to hours followed spring rain events in one case. Long-lived increases in nitrate and nitrite followed. N:P ratios fluctuates on a scale of days. Chlorophyll increases followed these runoff events but showed no relationship to nutrient data collected twice a week.

Monitoring HABs

- **Comparison of PNA vs DNA Probes in a Sandwich Hybridization Assay Format by Laurie Connell et al.** One of the new tools being developed for molecular analysis of HABs is the peptide nucleic acid, PNA, a DNA analog. PNA signal probes were more sensitive than the DNA signal probe. Deciding whether to use the PNA or the DNA probe must still be made on a case by case basis.
- **Remote Detection of HAB Species using the Environmental Sample Processor (ESP): Progress and Future Directions by Chris Scholin et al.** The Environmental Sample Processor is a new instrument being developed to allow real-time analysis for an extended period of time for HABs and the substances they produce. It collects water, concentrates microorganisms and automates the application of DNA probes (or other molecular probes). Results are transmitted in near-real time to shore for processing,

interpretation and dissemination. It also archives samples for nucleic acid, microscopic and toxin analyses in order to verify the near-real time analysis.

- **Application of Molecular Probes for the Detection of Harmful Algae on DNA-microchips by Linda Medlin et al.** They have developed a hand-held DNA microchip reader that can reliably detect harmful algae in field samples. The device costs about 25 Euros and it would be conceivable to custom design the chip to local HAB needs.
- **Application of Molecular Probes in Studies of *Alexandrium* in the Gulf of Maine: Successes and Problem Areas by Don Anderson et al.** “Molecular probes are useful tools for HAB research and monitoring, but care must be taken in their application and interpretation.”

Others:

- **Toxic Dinoflagellate Behaviour in a Southeast Tasmanian Estuary, Australia by Naomi Parker, et al.** The toxic dinoflagellate, *Gymnodinium catenatum*, causes periodic closures of shellfish farms. Bloomed in summer and autumn and exhibited vertical migration. Exhibited sexual reproduction and formed resting cysts. Anoxia and low light inhibit excystment.
- **Discovery of the Origin of Azaspiracids by Takeshi Yasumoto, et al.** Azaspiracids (AZAs) were first identified in 1995 when poisoning in the Netherlands occurred after people ate mussels harvested in Ireland. Since then there have been sporadic outbreaks in Ireland and parts of Europe. AZA has caused multiple organ injuries and lung tumor formation in mice receiving repeated oral doses of AZA. AZA is believed to be formed by the alga, *Protoperdinium crassipes*.
- **Detection and Identification of Paralytic Shellfish Poisoning Toxins in Florida Pufferfish Responsible for Incidents of Neurologic Illness by Michael Quilliam et al.** “Since January 2002, several incidents of human illness after eating pufferfish caught in waters near Titusville, Florida, had been reported.” The illnesses showed neurological symptoms. Three paralytic shellfish toxins, all different forms of saxitoxins, were found. The source of the toxin is not known.
- **Harmful Algal Blooms in South Carolina Ponds Associated with Housing and Golf Courses by Susan Wilde et al.** 37 of 45 brackish to marine man-made ponds that were sampled had HABs and in some cases ponds had multiple HAB species. These ponds were originally designed as buffers (receiving rainfall runoff from golf courses etc.) or for aesthetic purposes but because they receive elevated levels of nutrients and have restricted exchange, these ponds tend to promote development of HABs.
- **Black Water incident off west Florida coast:** probably resulted when a winter diatom (*Rhizosolenia*) bloom occurred over a decaying red tide and in an area of high carbonaceous dissolved oxygen material.
- **Pufferfish Poisoning: Widespread Implications of Saxitoxin in Florida by Jan Landsberg et al.** 19 cases this year of human poisoning from the consumption of pufferfish. Toxicity caused by high levels of saxitoxin in muscle, skin and mucus. Source of saxitoxin not known. One suspect is *Pyrodinium bahamense* var. *bahamense*.